Cohesion between sister chromatids must be established during DNA replication

Frank Uhlmann and Kim Nasmyth

Background: Cohesion between sister chromatids, which opposes the splitting force exerted by the mitotic spindle during metaphase, is essential for their segregation to opposite poles of the cell during anaphase. In *Saccharomyces cerevisiae*, cohesion depends on a set of chromosomal proteins called cohesins, which include structural maintenance of chromosomes 1p (Smc1p), Smc3p and sister chromatid cohesion 1p (Scc1p). Strains with mutations in the genes encoding these proteins separate sister chromatids prematurely and fail to align them in metaphase. This leads to missegregation of chromosomes in the following anaphase.

Results: In a normal cell cycle, Scc1p was synthesized and recruited to chromosomes at the onset of S phase. Using cells that expressed Scc1p exclusively from a galactose-inducible promoter, we showed that if Scc1p was synthesised only after completion of S phase, it still bound to chromosomes but failed to promote sister chromatid cohesion.

Conclusions: Cohesion between sister chromatids must be established during DNA replication, possibly following the passage of a replication fork. Furthermore, Scc1p (and other cohesins) are needed both for maintaining cohesion during mitosis and for establishing it during S phase. Establishment of sister chromatid cohesion is therefore an essential but hitherto neglected aspect of S phase.

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Received: **25 August 1998**
Revised: **8 September 1998**
Accepted: **8 September 1998**

Published: **18 September 1998**

Current Biology 1998, 8:1095–1101
http://biomednet.com/elecref/0960982200801095

Background

The establishment of sister chromatid cohesion and its subsequent dissolution after spindles attach to sister kinetochores is crucial for the faithful segregation of sister chromatids to opposite poles of the cell during anaphase [1–3]. A similar strategy might even be used by bacteria, in which transient cohesion between newly replicated origins might be important for their subsequent segregation [4]. Cohesion between sister chromatids in eukaryotes defines that they and not homologous chromatids are segregated from each other. If cohesion was merely established between two homologous chromatids during G2 phase, multicellular diploid organisms would all be chimaeras. One potential mechanism by which cohesion is confined to sister chromatids is that it is only generated between homologous DNA molecules emerging from a replication fork. An alternative mechanism is that cohesion is established at sites where sister chromatid molecules are still catenated following the collision of neighbouring forks [5,6].

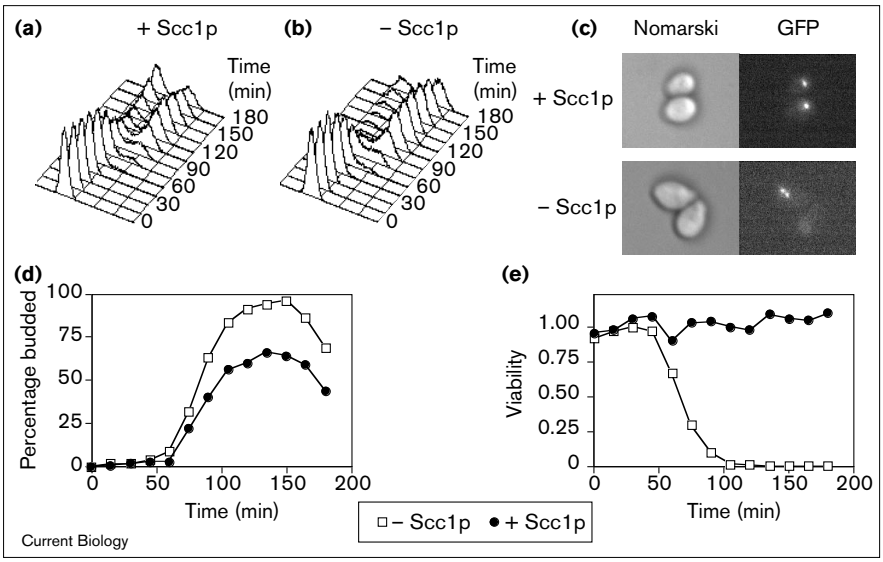
Known cohesion proteins fit into two categories: those like structural maintenance of chromosomes 1p (Smc1p), Smc3p and sister chromatid cohesion 1p (Scc1p) are bound to chromosomes throughout interphase in yeast [2] and *Xenopus* [7] and could therefore be involved in the establishment of cohesion, whereas the unrelated *Drosophila* protein MeiS322 binds to chromosomes when cells enter mitosis and may be required only to maintain cohesion [8]. We address here at which point during the cell cycle Scc1p must act: whether it must be present during DNA replication or whether it can still promote cohesion during G2, as might be possible if cohesins recognize the catenation holding sister chromatids together [9–11].

Results

Cells lose viability as they pass through S phase without Scc1p

In yeast, Scc1p (also called Mcd1p [3]) is an unstable protein whose levels fluctuate during the cell cycle. Protein synthesised in late G1 phase binds chromosomes throughout S and G2 and is rapidly degraded as it dissociates from chromosomes at the metaphase to anaphase transition [2]. The instability of Scc1p enabled us to manipulate its accumulation during the cell cycle using a strain in which Scc1p is synthesised exclusively from the galactose-inducible *GAL1-10* promoter. Small, unbudded G1 cells lacking Scc1p were isolated by centrifugal elutriation (from a culture pre-grown for 90 minutes in the absence of galactose) and incubated either in the absence of galactose (without Scc1p) or in the presence of galactose (with Scc1p). Cells from both cultures replicated their DNA (Figure 1a,b) and formed bipolar mitotic spindles

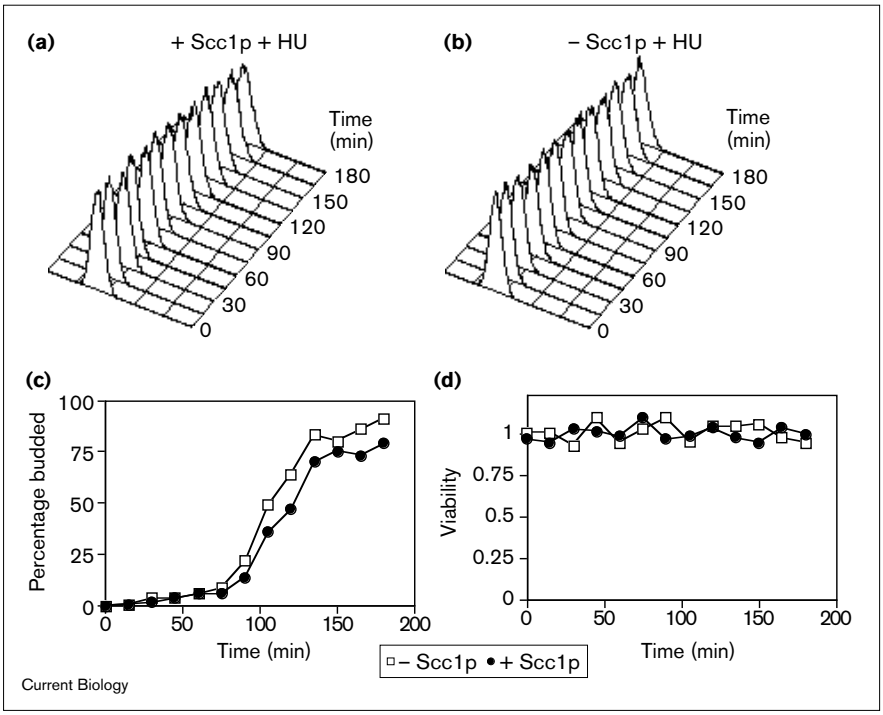
Figure 1



Viability is lost as cells pass through S phase without Scc1p. **(a)** The DNA content of Scc1p-depleted, unbudded G1 cells isolated by elutriation and released into YEP medium containing raffinose and galactose at 25°C (+ Scc1p). **(b)** As (a), but cells were released into YEP raffinose (– Scc1p). **(c)** Chromosome V was visualized with Tet–GFP in daughter cells at 180 min in cultures with or without Scc1p expression. 43% of unbudded daughter cells from the culture without Scc1p contained none or two GFP dots (21% contained no GFP dot and 22% contained two dots); 57% of the daughter cells contained one GFP dot. **(d)** The budding index of the cultures in (a), + Scc1p, and (b), – Scc1p. **(e)** A timecourse of the viability of cells released into medium containing galactose (+ Scc1p) or lacking galactose (– Scc1p).

with similar kinetics (data not shown), but only cells with Scc1p managed to segregate sister chromatids to opposite poles. Sister chromatids of chromosome V were visualized using a fusion protein between the tetracyclin repressor and green flourescent protein (Tet–GFP) that bound to tetracyclin operator sequences close to the chromosome's centromere (CenV) [2]. Cells that progressed through the cell cycle in the absence of Scc1p separated sister chromatids prematurely (data not shown), failed to segregate them to opposite poles and frequently produced daughter cells containing none or both of the sister chromatids (Figure 1c). Cells lacking Scc1p were also delayed in

Figure 2



Viability is rescued by blocking replication as cells enter S phase. **(a)** The DNA content of Scc1p-depleted, unbudded G1 cells isolated by elutriation and released into YEP medium containing raffinose, galactose and 100 mM HU at 25°C (+ Scc1p + HU). **(b)** As (a), but cells were released into YEP raffinose containing 100 mM HU (– Scc1p + HU). **(c)** The budding index of the cultures in (a), + Scc1p, and (b), – Scc1p. **(d)** A timecourse of the viability of cells released into medium with HU, containing galactose (+ Scc1p) or lacking galactose (– Scc1p).

undergoing cytokinesis, which explains why the fraction of budded cells transiently reached a higher level (Figure 1d). These phenotypes resemble those of temperature sensitive *scc1* mutants incubated at the restrictive temperature [2].

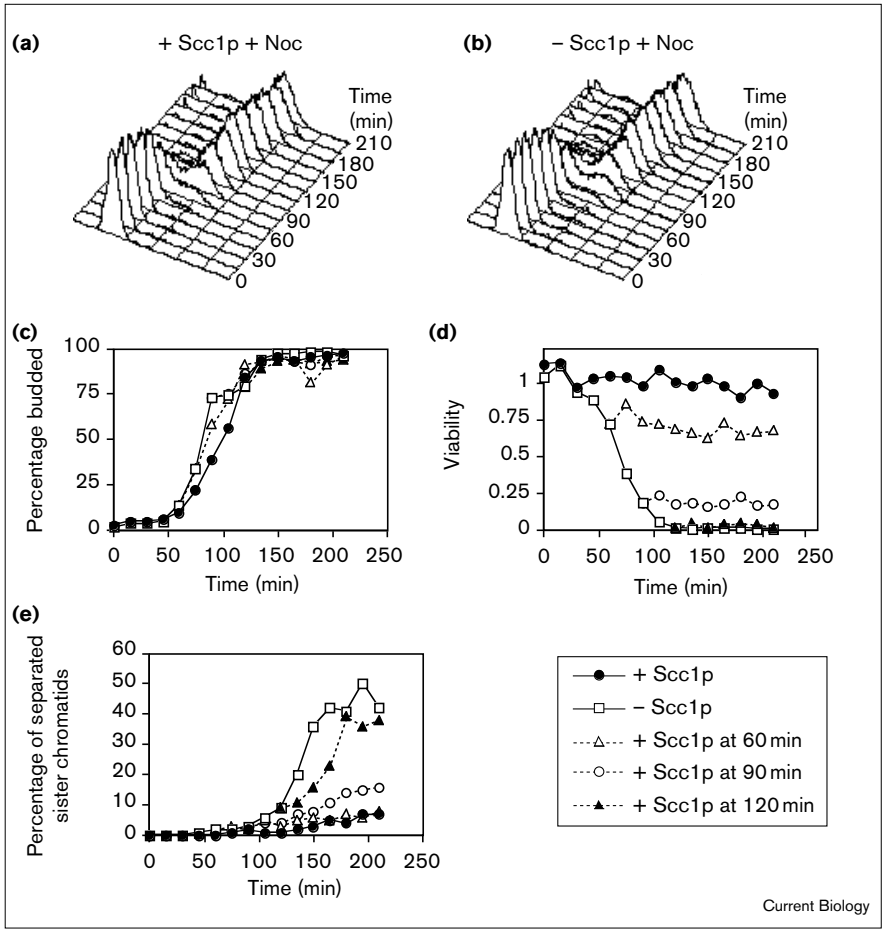
To test at what point during the cell cycle Scc1p was needed, we followed the viability of cells progressing through the cell cycle in the absence of Scc1p. Remarkably, all the cells grown in raffinose (and therefore not expressing Scc1p) lost viability with the same kinetics as they completed DNA replication (Figure 1e). Unbudded cells that had not yet started DNA replication were viable when plated in the presence of galactose but those that had budded and completed DNA replication were not. This implies that Scc1p is needed during S phase and can no longer function properly if made during G2.

Blocking DNA replication rescues viability of cells in S phase

To address whether DNA replication in the absence of Scc1p causes loss of viability during S phase, we incubated cells with and without Scc1p in the presence of hydroxyurea (HU). HU blocks DNA replication (Figure 2a,b), but not the activation of Cdk1–cyclin B kinases (data not shown), budding (Figure 2c), or the formation of mitotic spindles (data not shown). The loss of viability of cells grown without Scc1p in raffinose was completely suppressed by the addition of HU (Figure 2d).

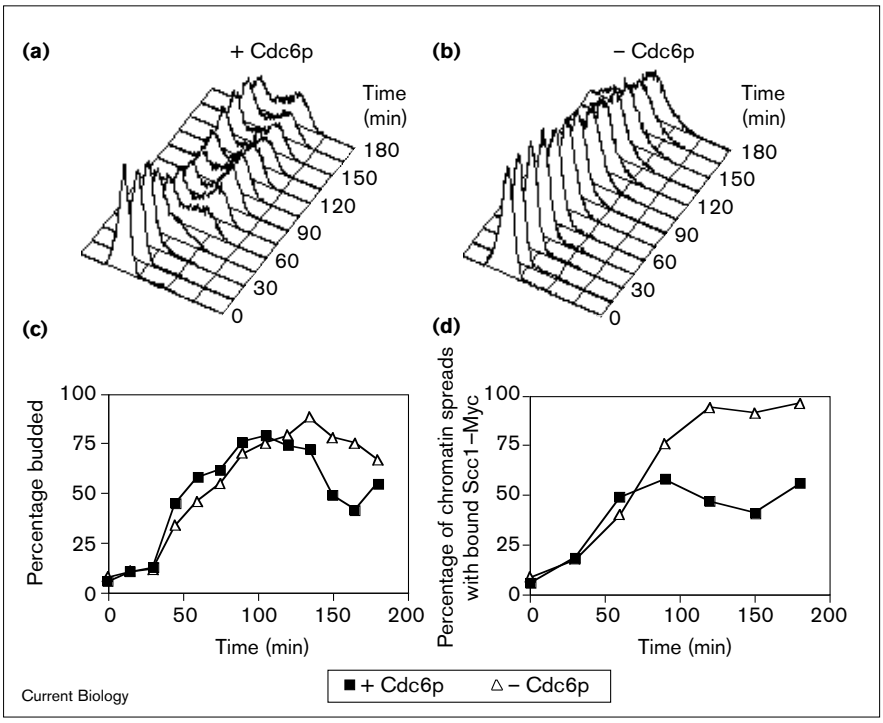
The depletion of deoxynucleotides induced by HU not only blocks DNA synthesis but also triggers a surveillance mechanism (checkpoint) that prevents the onset of anaphase [12]. To exclude the possibility that cells lacking Scc1p are rescued by HU because they no longer attempt sister chromatid separation, we incubated cells with and without Scc1p in the presence of nocodazole. Nocodazole does not affect DNA replication (Figure 3a,b) but by disassembling spindle microtubules it activates another surveillance mechanism that also inhibits sister chromatid separation. G1 cells were released into the cell cycle in five separate cultures in which Scc1p synthesis was induced by adding galactose at the timepoints indicated in Figure 3. The budding index and the viability of the cells in each culture were determined at intervals throughout the incubation period (Figure 3c,d). In contrast to HU, nocodazole failed to suppress the loss of viability of cells without Scc1p (Figure 3d), even though it prevented

Figure 3



Sister chromatids fail to align in cells undergoing S phase without Scc1p. **(a)** The DNA content of Scc1p-depleted, unbudded G1 cells isolated by elutriation and released into YEP medium containing raffinose, galactose, 15 μg/ml nocodazole (Noc) and 1% dimethylsulfoxide (DMSO) at 25°C. **(b)** As (a), but cells were released into YEP containing raffinose, nocodazole and DMSO. **(c)** The budding index as cells were released into either YEP containing raffinose, galactose, 15 μg/ml nocodazole and 1% DMSO (+ Scc1p); YEP containing raffinose, nocodazole and DMSO (– Scc1p); or YEP containing raffinose, nocodazole and DMSO when 2% galactose was added after 60 min, 90 min or 120 min. **(d)** The viability of cells in aliquots taken from the cultures described in (c) after diluting in 1% DMSO and plating onto YEP containing raffinose and galactose. **(e)** The percentage of cells, taken from the cultures described in (c), in which sister chromatids of chromosome V were not aligned, as visualized by the appearance of two separated fluorescent GFP dots in one cell body.

Figure 4



The association of Scc1–Myc with chromosomes in the absence of DNA replication. **(a)** The DNA content of cells released into YEP containing raffinose and galactose (+ Cdc6p). The cell cycle proceeds normally as Cdc6p is induced. **(b)** The DNA content of cells released into YEPD (– Cdc6p). Cells fail to initiate DNA replication and at later timepoints start to undergo 'reductional anaphase' [14]. **(c)** The budding index of the cultures in (a) and (b). **(d)** The percentage of cells with chromosome-associated Scc1–Myc as seen on chromosome spreads.

cytokinesis (Figure 3c) and re-replication (Figure 3a,b). Thus, both the kinetics of viability loss and its suppression by HU but not by nocodazole suggest that the viability loss is caused by DNA replication in the absence of Scc1p and not caused by another cell cycle event that also occurs during S phase. Unlike HU treatment, inactivation of topoisomerase II using a temperature-sensitive mutant (*top2-5* [10]) did not suppress loss of viability during S phase in the absence of Scc1p. Thus, Scc1p cannot establish cohesion in G2 at sites of sister chromatid intercatenation (data not shown).

Addition of galactose at various times to the culture grown in raffinose and nocodazole prevented any further reduction in the plating efficiency of the cells (Figure 3d). This shows that cells grown in liquid and solid medium respond to the re-synthesis of Scc1p in a similar manner. It also demonstrates that the lack of suppression by nocodazole is not due to the drug itself causing a loss of viability.

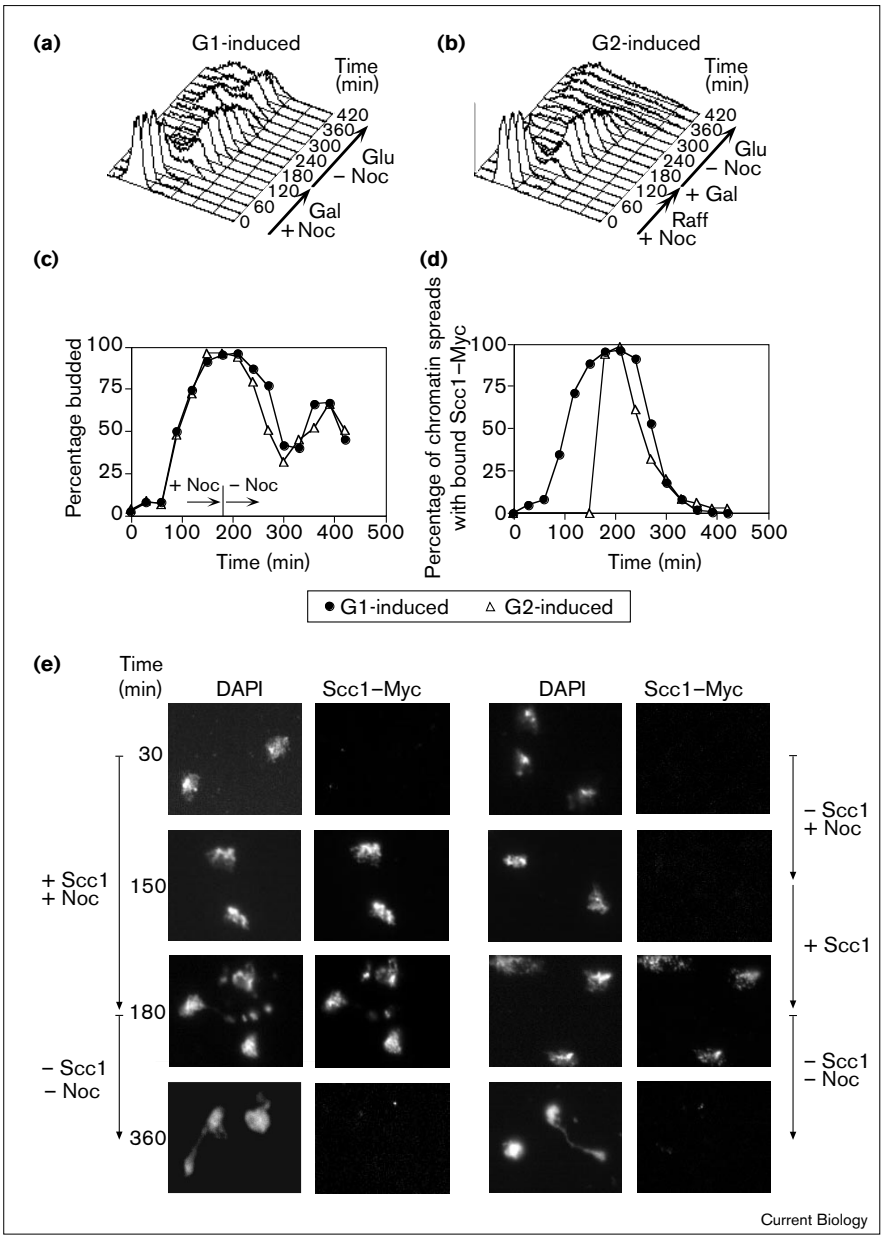
Cells undergoing S phase without Scc1p fail to align sister chromatids

To address the cause of viability loss we then assayed sister chromatid cohesion in each of the five nocodazole arrested cultures that differed only in the timing of Scc1p induction by galactose (Figure 3e). Erroneous separation of sister chromatids was detected by the appearance of two separated GFP dots, corresponding to CenV. At the end of the observation period (210 minutes), erroneous separation of sister chromatids was seen in no more than 5% of cells that received galactose from the beginning or at 60 minutes (that is, before S phase), whereas it was seen in over 40% of cells that received galactose at 120 minutes, when most cells had completed DNA replication. These results suggest that synthesis of Scc1p during G2 is ineffective in promoting sister chromatid cohesion. Cells lost viability faster than they lost the ability to hold together CenV sequences (compare Figure 3d and 3e). This is expected if inviability arises due to the loss of any one of the 16 yeast chromosomes.

Scc1p associates with chromosomes in the absence of DNA replication

One explanation for the inability of Scc1p to promote cohesion when it is expressed in G2 is that it might only be able to associate with chromosomes during DNA replication. To test this, we analysed (using chromosome spreads [2,13]) the association of Scc1p tagged with the Myc epitope (Scc1–Myc) with chromosomes in cells whose sole source of the replication initiator protein Cdc6p was under control of the *GAL* promoter [14]. Unbudded G1 cells depleted of Cdc6p were isolated by elutriation and incubated in the presence or absence of galactose (Figure 4a–c). Scc1–Myc associated with replicating (Figure 4a) and non-replicating (Figure 4b) chromosomes concomitantly with budding (Figure 4c,d). Thus, Scc1–Myc can bind to chromosomes in the absence of DNA replication. Similar observations have been made in the *Xenopus* cell-free system [7].

Figure 5



The association of Scc1–Myc with chromosomes in G2 without cohesion formation. **(a)** The DNA content of Scc1–Myc-depleted G1 cells isolated by elutriation and released into YEP medium containing raffinose, galactose, 15 μg/ml nocodazole and 1% DMSO at 25°C (Gal, + Noc). At 180 min cells were collected by filtration, washed with YEP containing raffinose, glucose and 1% DMSO, and resuspended in the same medium for further incubation at 25°C (Glu, – Noc). Using this protocol Scc1–Myc was induced as G1 cells were released into the cell cycle until the nocodazole arrest in metaphase, and was repressed as cells were released from the metaphase arrest (G1-induced). **(b)** As in (a), but cells were initially released into YEP containing raffinose, nocodazole and DMSO (Raff, + Noc). At 150 min, 2% galactose was added (+ Gal). At 180 min cells were transferred to YEP containing raffinose and glucose as in (a) (Glu, – Noc). Using this protocol, Scc1–Myc was expressed in G2 only during nocodazole arrest (G2-induced). **(c)** The budding index of the cultures in (a), G1-induced, and (b), G2-induced. **(d)** The percentage of cells with chromosome-associated Scc1–Myc as seen on chromosome spreads from cells in (a), G1-induced, and (b), G2-induced. **(e)** Examples of the chromosome spreads in (d). DNA was stained with DAPI and Scc1–Myc was detected with the anti-Myc monoclonal antibody 9E10 and Cy3 coupled secondary anti-mouse antibody as described [2].

Scc1p associates with chromosomes in G2 phase but fails to establish cohesion

To investigate whether Scc1p can bind to chromosomes when it is made during G2, we used a strain whose only source of Scc1p was Scc1–Myc expressed from the *GAL* promoter. Unbudded G1 cells depleted of Scc1–Myc were isolated by elutriation and incubated in the presence of nocodazole with or without galactose. Galactose was added to the latter culture at 150 minutes to induce Scc1–Myc expression when most cells had completed DNA replication. At 180 minutes, both cultures were transferred to glucose medium lacking nocodazole, in which further Scc1–Myc synthesis was repressed while cells were released from the nocodazole-induced arrest (Figure 5a–c). We monitored the accumulation of Scc1–Myc within cells by whole cell *in situ* immunofluorescence and monitored the binding of Scc1–Myc to chromosomes using chromosome spreads [2,13]. Scc1–Myc accumulated to high levels within nuclei within 30 minutes of its induction in all cells (see Supplementary material published with this paper on the internet), but little or no Scc1–Myc associated with chromosomes during early G1. Scc1–Myc associated with chromosomes in S, G2 and M phase cells, however, irrespective of whether its synthesis had begun in G1 or only in G2 (Figure 5d,e). Furthermore, Scc1–Myc dissociated from chromosomes

when cells attempted anaphase (following release from nocodazole) irrespective of whether it had associated with chromosomes during S phase or only during G2 (Figure 5d,e). As expected, Scc1–Myc made during G2 was dysfunctional. Instead of producing cells with a 1C DNA content upon cytokinesis (Figure 5a), these cells gave rise to cells with less than or more than 1C DNA content (Figure 5b).

These results imply that the association of Scc1–Myc with chromosomes is cell-cycle regulated. Scc1–Myc is incapable of binding chromosomes during early G1 despite accumulating within nuclei. At some point in late G1 or early S phase, Scc1p acquires the ability to bind chromosomes and maintains this property until the onset of anaphase, when it dissociates from chromosomes irrespective of whether or not it was at the time participating in sister chromatid cohesion.

Discussion

Our finding that Scc1p must be present during DNA replication suggests that it participates in a very early step in the establishment of sister chromatid cohesion. Scc1p is also required throughout G2 and M phases to maintain cohesion [2]. Because the binding of Scc1p to chromosomes depends on Smc1p [2], it is likely that Smc1p also participates in the establishment of cohesion. Our results also suggest that cohesion is not established by the recognition of sister chromatid catenation. Scc1p forms a complex with Smc1p and Smc3p, at least when not bound to chromosomes [3] (R. Ciosk, M. Shirayama and K.N., unpublished observations). This 'cohesin' complex seems capable of binding chromosomes for much of the cell cycle but it only forms 'joints' between sister chromatids when present during DNA replication. The formation of joints between sister chromatids is clearly an important feature of S phase. To investigate the structure of these joints and how they are formed in the context of DNA replication will, however, require a direct assay to measure them.

Chromosome loss during mitosis is thought to contribute to the malignancy of many tumours [15,16]. Whether defects in sister chromatid cohesion contribute to malignancy has not yet been established, but they certainly might contribute to the high rates of aneuploidy arising during meiosis in human females. Oocytes spend long periods arrested in G2 of the first meiotic division, a stage where sister chromatid cohesion is potentially vulnerable. An unequal chromosome complement in the second meiotic metaphase has been shown to result mainly from extra chromatids that separate from their sisters prematurely during the first meiotic division [17]. Our discovery that cohesion between sister chromatids cannot be repaired during G2 could explain why incidences of trisomies correlate with ageing. It might also explain why so few quiescent cells in animals arrest at this 'dangerous' stage of the cell cycle.

Conclusions

Scc1p is an essential sister chromatid cohesion protein whose association with chromosomes is cell cycle regulated. Our finding that Scc1p made during G2 phase fails to promote sister chromatid cohesion despite being able to bind chromosomes implies that cohesins bind to chromosomes in at least two modes. Only when cohesins are present during DNA replication can they form special joints between sister chromatids. The establishment of sister chromatid cohesion is therefore an integral aspect of S phase. An implication is that once sister chromatid cohesion is lost in G2, it cannot be rebuilt.

Materials and methods

Yeast strains

To create a strain expressing Scc1p exclusively under control of the *GAL* promoter, the Scc1p coding sequence was cloned with the *GAL1-10* promoter into a YIplac-derived vector [18] and integrated into the genome of strain K7204 at the *LEU2* locus. K7204 is a W303 derivative that expresses a GFP–tetracyclin repressor fusion protein and contains a cluster of tetracyclin operator sequences at the *URA3* locus as described [2]. The endogenous copy of the *SCC1* gene was then deleted by replacement with the *TRP1* gene yielding strain K7289. A strain expressing Scc1–Myc under control of the *GAL1-10* promoter (K6626) is described [2]. The endogenous copy of the *SCC1* gene in this strain was deleted by replacement with the *URA3* gene to yield strain K7062. The yeast strain K4675 expresses ubi*CDC6* under control of the *GAL* promoter as the only source of Cdc6p, as described [12]. The endogenous *SCC1* gene in this strain was tagged with 18 Myc epitopes in tandem by crossing with strain K6566 containing the tagged *SCC1* gene [2]. A diploid version of the resulting strain (K7324) was used for the experiment.

Media

YEP containing raffinose is complete medium (1% yeast extract, 2% peptone, 55 mg/l adenine) with 2% raffinose as a carbon source. Either galactose or glucose were added to this medium at 2% concentration. YEPD is complete medium containing 2% glucose but no raffinose.

Cell cycle experiments

Strains were grown at 25°C in YEP containing raffinose and galactose until mid-log phase. Cells were collected by filtration, washed with YEP raffinose, and grown for further 90 min in YEP raffinose at 25°C to deplete cells of Scc1p. When strain K7324 was used, growth in YEP raffinose was for 75 min to deplete cells of Cdc6p. Then cells were harvested by centrifugation, and small, unbudded G1 cells were isolated by centrifugal elutriation at 4°C. G1 cells were released into the cell cycle by diluting them into medium at 25°C, containing or lacking galactose to induce expression of the respective genes controlled by the *GAL* promoter.

Viability assay

The viability of cells was measured by diluting aliquots of the cultures at the indicated timepoints and plating a constant volume of two serial dilutions (equal to 3000 and 300 cells, respectively) onto YEP plates with raffinose plus galactose. Plates were incubated at 30°C and the number of colonies grown was counted after 2 days The increase in cell number by cell division during the course of the experiment was estimated from the decrease in budding index as cytokinesis occurs and was taken into account when calculating viability.

Other techniques

FACScan analysis for DNA content, visualization of CenV using Tet–GFP, and chromosome spreads were performed as described [2].

Supplementary material
A figure showing examples of the whole cell *in situ* immunofluorescense used to monitor Scc1–Myc expression is published with this paper on the internet.

Acknowledgements

We thank C. Holm for *top2* mutant strains, R. Ciosk, C. Michaelis, M. Shirayama and A. Toth for communicating unpublished results and for yeast strains, M. Glotzer, J. Knoblich, and J.-M. Peters for critical reading of the manuscript. F.U. acknowledges support through an EMBO long-term fellowship.

References

1. Kerrebrock AW, Moore DP, Wu JS, Orr-Weaver TL: **Mei-S332, a *Drosophila* protein required for sister-chromatid cohesion, can localize to meiotic centromere regions.** *Cell* 1995, **83:**247-256.
2. Michaelis C, Ciosk R, Nasmyth K: **Cohesins: chromosomal proteins that prevent premature separation of sister chromatids.** *Cell* 1997, **91:**35-45.
3. Guacci V, Koshland D, Strunnikov A: **A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *MCD1* in *S. cerevisiae*.** *Cell* 1997, **91:**47-57.
4. Lin DCH, Grossman AD: **Identification and characterization of a bacterial chromosome partitioning site.** *Cell* 1998, **92:**675-685.
5. Sundin O, Varshavsky A: **Arrest of segregation leads to accumulation of highly intertwined catenated dimers: dissection of the final stages of SV40 DNA replication.** *Cell* 1981, **25:**659-669.
6. Murray AW, Szostak JW: **Chromosome segregation in mitosis and meiosis.** *Annu Rev Cell Biol* 1985, **1:**289-315.
7. Losoda A, Hirano M, Hirano T: **Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion.** *Genes Dev* 1998, **12:**1986-1997.
8. Moore DP, Page AW, Tang TTL, Kerrebrock AW, Orr-Weaver TL: **The cohesion protein Mei-S322 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate.** *J Cell Biol* 1998, **140:**1003-1012.
9. DiNardo S, Voelkel K, Sternglanz R: **DNA topoisomerase II mutant of *Saccharomyces cerevisiae*: topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication.** *Proc Natl Acad Sci USA* 1984, **81:**2616-2620.
10. Holm C, Goto T, Wang JC, Botstein D: **DNA topoisomerase II is required at the time of mitosis in yeast.** *Cell* 1985, **41:**553-563.
11. Shamu CE, MurrayAW: **Sister chromatid separation in frog extracts requires DNA topoisomerase II activity during anaphase.** *J Cell Biol* 1992, **117:**921-934.
12. Elledge SJ: **Cell cycle checkpoints: preventing an identity crisis.** *Science* 1996, **274:**1664-1671.
13. Klein F, Laroche T, Cardenas M, Hofmann J, Schweitzer D, Gasser S: **Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast.** *J Cell Biol* 1992, **117:**935-948.
14. Piatti S, Bšhm T, Cocker JH, Diffley JFX, Nasmyth K: **Activation of S-phase-promoting CDKs in late G1 defines a 'point of no return' after which Cdc6 synthesis cannot promote DNA replication in yeast.** *Genes Dev* 1996, **10:**1516-1531.
15. Lengauer C, Kinzler KW, Vogelstein B: **Genetic instability in colorectal cancers.** *Nature* 1997, **386:**623-627.
16. Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, *et al.*: **Mutations of mitotic checkpoint genes in human cancers.** *Nature* 1998, **392:**300-303.
17. Angell R: **First-meiotic-division nondisjunction in human oocytes.** *Am J Hum Genet* 1997, **61:**23-32.
18. Gietz RD, Sugino A: **New yeast–*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base-pair restriction sites.** *Gene* 1988, **74:**527-534.

Supplementary material

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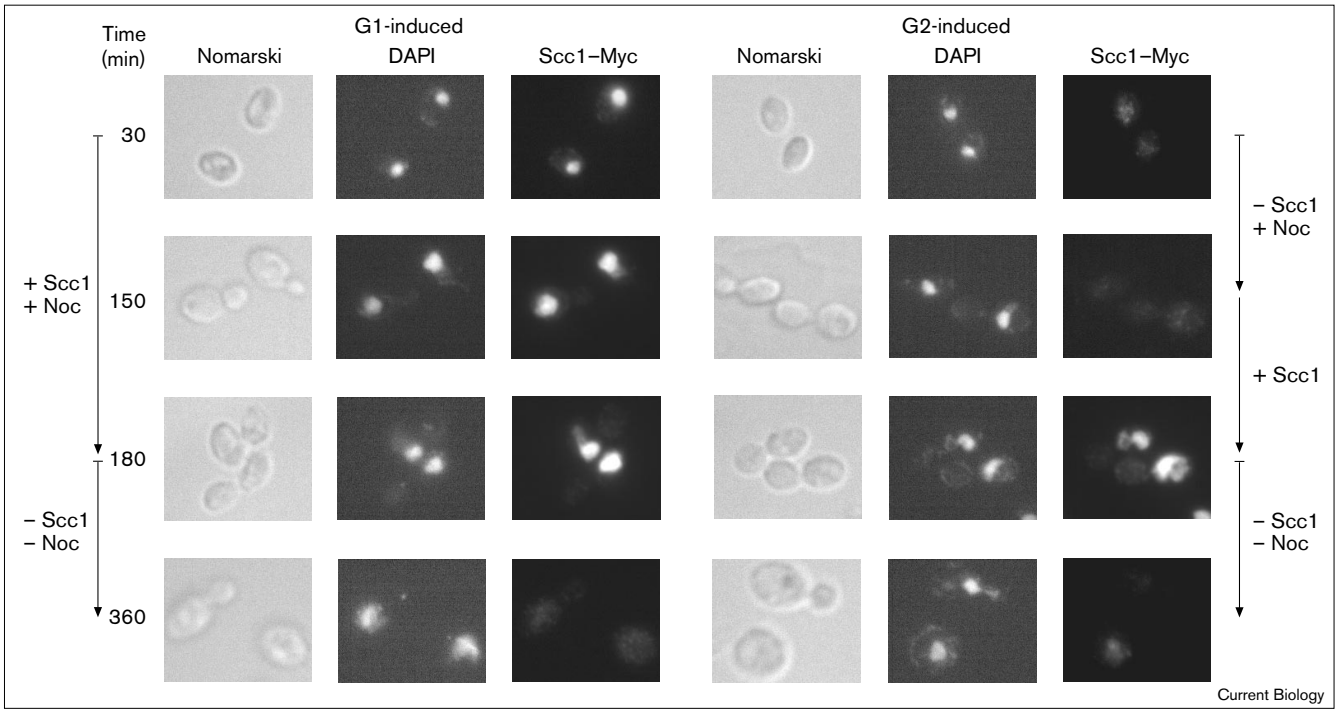
Current Biology 18 September 1998, **8**:1095–1101

Figure S1



Whole cell *in situ* immunofluorescence to monitor Scc1–Myc expression. Examples are shown for each of the timepoints presented in Figure 5e. Cell shape was seen in Nomarski interference contrast. Nuclear DNA was stained with DAPI. Scc1–Myc was detected with the anti-Myc monoclonal antibody 9E10 and a Cy3-coupled secondary anti-mouse antibody [2].